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The Effects of Salinity Change on the Free Amino Acid Pools of Two Nereid Polychaetes, 'Neanthes succinea' and 'Leonereis culveri'

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16. ABSTRACT

The response of the free amino acid pools of two nereid polychaetes, Neanthes succinea and Leonereis culveri to both increased and decreased salinities was examined. In both species, glycine and alanine accounted for most of the observed change in the total free amino acid (FAA) pool size.

Glutamine values increased under hypo-osmotic conditions suggesting that this amino acid plays a role in nitrogen storage during the reduction in FAAs which occurs with reduced salinity. The salinity response was superimposed on a "laboratory stress" response characterized by a generalized increase in the FAA pool size. The two responses appeared to be additive in nature. Significant differences in the responses of individual amino acids between these two species indicates that interspecific variation exists in the response to salinity change in nereid polychaetes.

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THE EFFECTS OF SALINITY CHANGE ON THE FREE AMINO ACID POOLS OF TWO NEREID POLYCHAETES, *NEANTHES SUCCINEA* AND *LEONEREIS CULVERI*

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Abstract—1. The response of the free amino acid pools of two nereid polychaetes, *Neanthes succinea* and *Laeonereis culveri* to both increased and decreased salinities was examined. In both species, glycine and alanine accounted for most of the observed change in the total free amino acid (FAA) pool size.

2. Glutamine values increased under hypo-osmotic conditions suggesting that this amino acid plays a role in nitrogen storage during the reduction in FAAs which occurs with reduced salinity.

3. The salinity response was superimposed on a "laboratory stress" response characterized by a generalized increase in the FAA pool size. The two responses appeared to be additive in nature.

4. Significant differences in the responses of individual amino acids between these two species indicates that interspecific variation exists in the response to salinity change in nereid polychaetes.

INTRODUCTION

The tissues of marine invertebrates typically contain a greater concentration of free amino acids than do the analogous tissues of terrestrial and freshwater organisms (Kittredge *et al.*, 1962; Awapara, 1962; Jeffries & Alzara, 1970; Weber & Van Marrewijk, 1972; Gilles, 1979). One reason for this discrepancy is that in marine animals exposed to variation in salinity, free amino acids (FAA) play an important role as solutes in the establishment of an isosmotic balance between cells and the extracellular fluid. These small organic molecules along with cell electrolytes, can be mobilized to compensate for any changes that might occur in extracellular osmotic pressure. This "ion-sparing effect" (Dall, 1975) has been demonstrated in every euryhaline marine invertebrate studied so far (Gilles, 1979), some marine vertebrates (Jürss, 1980; King *et al.*, 1980), and in marine plants (Gilles & Pequeux, 1977). In all of these organisms, tissue levels of free amino acids have been found to vary almost linearly with external salinity, increasing under hyperosmotic conditions and decreasing under hypo-osmotic conditions.

Generally only a relatively small number of non-essential amino acids undergo significant changes in concentration (Gilles, 1978). The amino acids utilized, however, are not the same in all phyla. In muscle tissue of molluscs, for example, taurine is often present in the greatest concentration, and along with glycine and alanine, accounts for most of the intracellular response to change in extracellular fluid osmolarity (Pierce, 1971; Severin *et al.*, 1972; Baginski & Pierce, 1977; Shumway & Gabbot, 1977; Sansone *et al.*, 1978). Many crustaceans, on the other hand, seem to preferentially utilize glycine and/or proline in restoring osmotic balance, and these amino acids are characteristically most abundant (Severin *et al.*, 1972; Roesijadi *et al.*, 1976; Vivares *et al.*, 1980; Weber & Van Marrewijk, 1972; Cobb *et al.*, 1975).

Because gross similarities in the concentrations of free amino acids are apparent at the phylum level, it has been suggested that free amino acid patterns might prove to be a more reliable indication of phylogenetic relationship than the more typically studied morphological characteristics (Abati & Reish, 1972). Although insects have been classified to species based on similarities or differences in their tissue free amino acid levels (Ball & Clark, 1953; Micks & Gibson, 1957), this method of biochemical taxonomy has not been successfully applied to marine euryhaline invertebrates (Jeffries & Alzara, 1970). In fact, more often than not, rather substantial variability may be found in the free amino acid content of the same tissues taken from individuals of the same species acclimated at the same salinity (Forster & Hannafin, 1980). On the other hand, similar patterns of response to salinity stress might be expected among closely related species. The objective of this study was to compare the free amino acid response to salinity change in two closely related polychaete species in order to determine whether they utilize the same amino acids in the same ways to counter an osmotic imbalance. In addition, the effect of laboratory acclimation itself on the size and composition of the free amino acid pools of these two species was examined.

METHODS

Specimens of *Neanthes succinea* (*Nereis succinea*) were collected by sieve from shallow, near-shore regions of Christmas Bay, Texas. Salinity at the time of capture was 25 ppt. Specimens of *Laeonereis culveri* were taken at the eastern edge of Galveston Island near Galveston Inlet at a salinity of 19 ppt. Identifications are from Pettibone (1963).

Three individuals of each species were frozen on dry ice immediately after collection so that a quantitative determination of the free amino acid pool structure under natural conditions could be made. The remaining polychaetes were transported to the laboratory, placed in finger bowls with a

small amount of native sediment, and acclimated to laboratory conditions at the salinity of capture for 48 hr.

After 48 hr, three experimental groups were established for each species:

(1) a control group maintained at the salinity of capture.

(2) an experimental group exposed to a step-wise increase in salinity totaling 10 ppt above the salinity of capture over a period of 5 days.

(3) a third group subjected to a 10 ppt step-wise decrease in salinity by the same method.

The water was changed in all bowls every eight hr. Salinity changes were made every 24 hr in increments of two ppt. Salinities were increased by addition of "Instant Ocean" to seawater collected at the time of capture and decreased by addition of distilled water. All animals were maintained at room temperature under a 12 hr light:12 hr dark cycle.

After five days, four polychaetes from each treatment were pooled, frozen on dry ice and freeze-dried. Lyophilized dry weights were determined for the pooled sample. The samples were homogenized and centrifuged, Norleucine was added to the resulting supernatant as an internal standard. The supernatant was brought to a final volume of 10% trichloroacetic acid with 50% TCA. After 30 min the precipitated macromolecules were removed by centrifugation and the resulting supernatant extracted six times with diethyl ether to remove the TCA. The samples were then lyophilized and taken up in 0.1 N HCl.

Amino acid content was determined using an Aminco amino acid analyzer. Analysis of each sample required two and one-half hr using a sodium citrate buffer system with *o*-phthaldialdehyde (OPA) as the detecting compound. Because serine coelutes with asparagine and glutamine in our system, and because cysteine reacts poorly with OPA, a second aliquot was oxidized using the procedure outlined by Moore (1963) and then hydrolyzed in 2 N HCl for two hr at 100° C. Asparagine and glutamine were estimated by the resulting increase in aspartate and glutamate respectively, in the second aliquot relative to the first. Cysteine was estimated as cysteic acid. Proline, which could not be detected fluorometrically with OPA, and cysteine, lysine, arginine and histidine, which were present in trace amounts only, are not included in the estimates of pool size reported in this paper.

RESULTS

Of the 15 amino acids present in significant quantity, glycine and taurine were consistently the most abundant in the free amino acid pools of both species (Tables 1 and 2). These two compounds accounted for more than 60% of the free amino acids measured in *Neanthes succinea* and, except at the lowered salinity, for at least 45% of those measured in *Laeonereis culveri*. Other amino acids typically present in high concentrations included serine, alanine, and glutamine in *Neanthes succinea* and these three plus glutamic acid in *Laeonereis culveri*.

For both species, total pool size increased with an increase in salinity and decreased in similar fashion at reduced salinity. Of those amino acids that undergo significant changes in concentration in *Neanthes succinea*, glycine was clearly the most responsive to alterations in the osmotic environment. It accounted for 71.4% of the pool decrease under hypo-osmotic conditions and for over 100% of the increase under hyperosmotic conditions (Tables 1 and 2). Interestingly, all amino acids except glycine, alanine, glutamic acid, methionine and phenylalanine actually decreased with an increase in salinity. Thus, in the absence of glycine, *Neanthes succinea* could not have made an appropriate osmotic adjustment to increased salinity. At low salinity on the other hand, the concentration of all amino acids except taurine, aspartic acid and glutamine decreased.

The opposite appears to be true for *Laeonereis culveri*. With increased salinity, most of the amino acids increased and unlike *N. succinea*, alanine accounted for much of the increase. Lesser, but important, contributions were made by glycine, glutamic acid, asparagine and glutamine. The response of individual amino acids to hypo-osmotic conditions was quite varied, however, in contrast to the generalized loss noted in *N. succinea*. Glycine and alanine accounted for most of the total net reduction found, but one half of the glycine-alanine decrease was offset by a substantial increase in glutamine plus smaller increases in leucine, tyrosine, threonine, and valine.

The size of the free amino acid pool of animals kept under laboratory conditions and those frozen immediately upon collection in the field was markedly different (Table 3). The concentration of every free amino acid (except aspartate in *Laeonereis*) was greater in polychaetes kept in the laboratory than in those frozen on dry ice immediately after capture. This may indicate that in addition to the stress imposed by varying the salinity of the medium, both species maintained in the laboratory were subject to additional stress(es) not normally encountered in the field.

DISCUSSION

Nereid polychaetes are known to compensate for salinity alterations by effecting changes in the concentrations of both inorganic and organic solutes (Freel *et al.*, 1973; Abati & Reish, 1972; Jeuniaux *et al.*, 1961; Haberfield *et al.*, 1975). Both *Neanthes succinea* and *Laeonereis culveri* are able to compensate for salinity change by varying the concentrations of intracellular free amino acids. Marked differences exist, however, in the way that the free amino acid pools are mobilized in these two species. *N. succinea* depends almost entirely on glycine. Most of the increase in the FAA pool during hyperosmotic conditions and most of the decrease during hypo-osmotic conditions is caused by this amino acid. *L. culveri*, on the other hand depends on both glycine and alanine, with alanine more important under hyperosmotic conditions and glycine more important under hypo-osmotic conditions.

The metabolism of glutamine also differs between these two species. In *Laeonereis culveri*, glutamine increases dramatically at decreased salinity. The increase is much more modest in *Neanthes succinea*. Under hyperosmotic conditions, glutamine decreases in *N. succinea* but increases in *L. culveri*. Glutamine has been implicated in nitrogen storage and nitrogen transport in vertebrates (Chang & Goldberg, 1978; Goldberg & Chang, 1978). A nitrogen storage function may explain the significant increase in glutamine observed in *L. culveri* at decreased salinity. This increase balances out about 30% of the entire glycine-alanine decrease in this species.

Table 1. Response of free amino acids to decreased salinity

Amino acid	*Neanthes succinea* Concentration (μmol/g dry wt) 25‰	15‰	% Change	% Contribution to pool change	*Laeonereis culveri* Concentration (μmol/g dry wt) 19‰	9‰	% Change	% Contribution to pool change
Tau	89.26	97.48	+9.2	−4.6	107.65	99.26	−7.8	7.9
Asp	15.18	16.37	+7.8	−0.7	17.74	13.47	−24.1	4.0
Thr	20.06	12.60	−37.2	4.2	8.29	29.67	+258	−20.2
Ser	62.57	60.84	−2.8	1.0	28.25	30.21	+6.9	−1.8
Glu	22.12	19.29	−12.8	1.6	24.95	19.82	−20.6	4.8
Gly	403.98	276.86	−31.5	71.4	213.38	29.80	−86.0	173.2
Ala	57.92	42.72	−26.2	8.5	65.03	15.14	−76.7	47.1
Val	22.98	12.17	−47.0	6.1	8.11	23.99	+196	−15.0
Met	18.73	11.36	−39.3	4.1	7.57	8.17	+7.9	−0.6
Ile	15.18	11.26	−25.8	2.2	4.95	14.50	+193	−9.0
Leu	27.18	25.95	−1.2	0.7	12.62	29.98	+136	−16.4
Tyr	22.11	17.22	−22.1	2.7	11.40	22.80	+100	−10.8
Phe	13.98	9.38	−32.9	2.6	8.70	14.67	+68.6	−5.6
Asn	39.92	24.30	−39.1	8.8	26.40	20.98	−20.5	5.1
Gln	94.00	109.45	+16.4	−8.7	39.06	105.63	+170	−62.8
Total	925.17	747.25	−19.2	100.0	584.10	478.09	−18.1	100.0

Response of the free amino acid pool of *Neanthes succinea* and *Laeonereis culveri* to a decrease of 10‰ below ambient salinity.

Table 2. Response of free amino acids to increased salinity

Amino acid	*Neanthes succinea* Concentration (μmol/g dry wt) $25^{o}/_{oo}$	$35^{o}/_{oo}$	% Change	% Contribution to pool change	*Laeonereis culveri* Concentration (μmol/g dry wt) $19^{o}/_{oo}$	$29^{o}/_{oo}$	% Change	% Contribution to pool change
Tau	89.26	78.73	−11.8	−8.61	107.65	113.54	+5.5	3.25
Asp	15.18	15.01	−1.1	−0.14	17.74	14.84	−16.3	−1.60
Thr	20.06	18.07	−9.9	−1.63	8.29	14.42	+73.9	3.38
Ser	62.57	61.65	−1.5	−0.75	28.25	36.73	+30.0	4.67
Glu	22.12	26.72	+20.8	3.76	24.95	42.64	+70.9	9.75
Gly	403.98	565.82	+40.1	132.34	213.38	239.51	+12.2	14.40
Ala	57.92	73.64	+27.1	12.85	65.03	147.29	+126.5	45.34
Val	22.98	14.70	−36.0	−6.77	8.11	18.25	+125.0	5.59
Met	18.73	22.71	+21.2	3.25	7.57	7.17	−5.3	−0.22
Ile	15.18	12.96	−14.6	−1.82	4.95	7.51	+51.7	1.41
Leu	27.18	25.78	−5.2	−1.14	12.62	12.27	−2.8	−0.19
Tyr	22.11	19.55	−11.6	−2.09	11.40	8.61	−24.5	1.54
Phe	13.98	14.50	+3.7	0.43	8.70	9.89	+13.7	0.65
Asn	39.92	24.12	−39.6	−12.92	26.40	39.93	+51.2	7.46
Gln	94.00	73.50	−21.8	−16.76	39.06	52.94	+35.5	7.65
Total	925.17	1047.46	+13.2	100.00	548.10	765.54	+31.1	100.00

Response of the free amino acid pool of *Neanthes succinea* and *Laeonereis culveri* to an increase of $10^{o}/_{oo}$ over ambient salinity.

Table 3. Concentrations of free amino acids in field and laboratory animals at the same salinities

Amino acid	*Neanthes succinea* Concentration (μmol/g dry wt) Laboratory	Field	*Laeonereis culveri* Concentration (μmol/g dry wt) Laboratory	Field
Tau	89.26	45.70	107.65	63.56
Asp	15.18	7.33	17.74	24.05
Thr	20.06	3.48	8.29	6.30
Ser	62.57	25.26	28.25	17.80
Glu	22.12	10.44	24.95	24.30
Gly	403.98	317.89	213.38	77.15
Ala	57.92	*	65.03	53.56
Val	22.98	3.72	8.11	4.73
Met	18.73	7.97	7.57	*
Ile	15.18	2.73	4.95	1.93
Leu	27.18	5.96	12.62	4.94
Tyr	22.11	5.12	11.40	*
Phe	13.98	4.78	8.70	5.12
Asn	39.92	18.25	26.40	0.82
Gln	94.00	20.45	39.06	16.23

The concentrations of free amino acids found in nereids collected in the field and those maintained in the laboratory under experimental conditions at field salinities.

* Value not determined.

Interspecific variability in other amino acids besides glycine, alanine, and glutamine can also be observed. Under decreased salinities, one expects a generalized decrease in the concentrations of most of the amino acids of the free amino acid pool. This is observed in *Neanthes succinea*. In *Laeonereis culveri*, however, 9 of the 15 amino acids measured actually increase. Increases in threonine, valine, leucine, tyrosine and glutamine are substantial. At high salinities, one expects a generalized increase in the amino acids of the free amino acid pool. This is the observed result in *L. culveri*, but, in *N. succinea*, ten of the measured amino acids actually decrease. Decreases in glutamine, asparagine and taurine are particularly important. For example, taurine responds as expected to salinity changes in *Laeonereis culveri*, decreasing in quantity at low salinity and increasing at high salinity. In *Neanthes succinea*, however, the response is just the opposite (Tables 1 and 2). Although some of the minor changes are probably due to sample variability, in many respects, the responses of *N. succinea* and *L. culveri* to salinity change are very dissimilar even though the two polychaetes are in the same family, Nereidae.

Differences in the salinity response can also be noted between the two species investigated here and other nereids previously studied by Abati & Reish (1972) and Jeuniaux *et al.* (1961). For example, in *Nereis diversicolor*, in addition to glycine, both alanine and glutamic acid decrease substantially during hypo-osmotic stress with aspartic acid while methionine and proline decrease to a lesser extent (Jeaniaux *et al.*, 1961). In *Perinereis cultrifera*, a glycine decrease was the dominant effect with less important decreases in alanine, glutamic acid, methionine, and threonine (Jeuniaux *et al.*, 1961).

Possibly, intraspecific variability in the free amino acid pool unrelated to osmotic conditions is sufficiently great to obscure interspecific similarities in the salinity response and explain the results noted above. Both Ellis (1937) and Fretter (1955) suggest that physiological races exist in *Nereis diversicolor*. In addition, other stresses unrelated to osmotic stress may affect the FAA pool. For example, both polychaete species acclimated to laboratory conditions have higher free amino acid levels at control laboratory salinities and in many cases higher free amino acid levels at lowered laboratory salinities than did those frozen at the control salinity in the field. If only laboratory animals are compared, however, the salinity response is still virtually linear and clearly superimposed on the free amino acid pool's responses generated by the laboratory conditions. Possible explanations for the increase in FAA in laboratory animals are starvation, the process of collection and transfer to the laboratory, overcrowding, or lack of sufficient sediment cover. Since amino acids *per se* seem to have no particular function in countering these various types of stress, it seems likely that the increased concentrations observed in the laboratory animals are merely incidental to the energy release accompanying protein catabolism. The pronounced increase in almost every free amino acid, including the essential ones supports this hypothesis. Jeffries (1972) and Squires *et al.* (1979) also noted changes in the free amino acid pools of animals subjected to laboratory conditions. Such results suggest caution in the application of laboratory data to field situations. *In situ* studies are clearly needed.

Stresses other than these introduced in the laboratory can also generate modification in the free amino acid pool. Pollution (Schafer, 1971; Jeffries, 1972; Roesijadi *et al.*, 1976), temperature changes (Jeffries, 1969; Jeffries & Alzara, 1970; Kasschau, 1975), parasitism (Feng *et al.*, 1970; Vivares *et al.*, 1980), low oxygen (Schafer, 1961; Felbeck & Grieshaber, 1980), and sex or reproductive state (Feng *et al.*, 1970; Ferguson, 1975; Roesijadi *et al.*, 1976) all influence the size and composition of the free amino acid pool. Thus, animals belonging to the same species but collected from different locations or at different times of the year may exhibit markedly different free amino acid patterns even if salinity differences are negligible (Mearns & Reish, 1969; Sansone *et al.*, 1978). When the above occur concurrently with an alteration in salinity, they may be expected to modify the salinity response (Kasschau, 1975; Roesijadi *et al.*, 1976).

If conservative metabolic approaches (homologous pathways in the sense of Florkin, 1966) to salinity change in the free amino acid pool are present within higher taxonomic groups of the invertebrates, their presence in the Nereidae is by no means clear. As in many of the invertebrates, glycine and alanine are important amino acids in the free amino acid pool's response to salinity change. One or both of these is important in every nereid studied to date, but no consistency in the degree in which these are used can be discerned. The dissimilarity in the degree of utilization of the amino acids of lesser importance is also substantial. Pierce (1971) noted a similar phenomenon in two species of *Modiolus* mussels. Variation in the salinity response, as noted here between *Neanthes succinea* and *Laeonereis culveri*, might be due to either differences in physiological state or to basic metabolic

differences in the organisms' strategy to counter salinity change. An understanding of the evolution of the osmotic response exhibited by the FAA pool requires the determination of whether these responses as described here are truly homologous or represent one or more convergences within the family Nereidae. The future elucidation of this question must depend on a much better understanding of the many other factors affecting the composition of the free amino acid pool.

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